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Risk of *Bacillus cereus* contamination in cooked rice

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Abstract

Rice is prone to contamination with *Bacillus cereus* which causes food poisoning. The aim of this study was to investigate the presence of *Bacillus cereus* in cooked and uncooked rice. A total of forty samples of rice were collected from local supermarkets and tested before and after cooking for the presence of *B. cereus.* Half of the uncooked rice samples were *B. cereus* positive. In contrast *B. cereus* was not detected in the cooked samples when they were immediately examined for the presence of *B. cereus*. Storing the cooked sample at room temperature (25 °C) for twelve hours, however, gave the spores the chance to grow, and two samples showed bacterial growth higher than 1×10^3 Cfu/g. The D-value of *B. cereus* ATCC 14579 spores was found to be 2.7, 1.55, 1.35, 1.2 and 0.6 min at 80, 85, 90, 95, and 100 °C respectively. The Z-value was 9 °C. The level of bacterial growth was below the limits but it was sufficient to suggest that cooked rice should be routinely checked for *B. cereus* to control bacterial contamination and reheating should reach 80 °C to insure the safety.

Keywords: *Bacillus cereus*; *D*-value; *Z*-value; cooked rice; uncooked rice; food poisoning; food safety.

Practical Application: Exposing cooked rice to a wide range of temperature during storage might support the multiplication of bacteria in contaminated food. This study to control the safety of both cooked and uncooked rice and reduce the risk of Bacillus cereus, storing temperature and routine checks are recommended.

1 Introduction

Rice (Oryza sativa) is one of the most important agricultural crops with approximately 489 million tons being consumed during last year (United States Department of Agriculture, 2020) and half the world's population eat rice on a daily basis (Sohn, 2014). According to the statistics, Saudi Arabia ranks sixth in rice imports, importing 1.1 million tons last year, a figure expected to reach 1.3 million tons in 2020 (United States Department of Agriculture, 2020) . In Saudi Arabia, rice is a national staple and is considered the main meal in all gatherings such as Eid, weddings and dinner parties. Most Saudis eat rice every day (Al Tamimi, 2016), and many popular dishes are rice based, such as Kabsa, Bukhari rice, Biryani, Mandi and Almefalf (Al-Kanhal et al., 1999). Basmati rice is the most requested kind and 78.7% of Saudi Arabia's rice imports come from India (United States Department of Agriculture, 2020).

In general, starchy foods such as rice are common vehicles for *Bacillus cereus,* which is a common cause of food poisoning (Murray et al., 2007; Glasset et al., 2016). Briefly, *B. cereus* is an aerobic and spore-forming bacteria (Ehling-Schulz et al., 2019), commonly found in the environment and able to grow in food (McDowell et al., 2020). Although the soil is considered its primary reservoir. *B. cereus* spores, or active or vegetative cells present in the intestines of insects might contaminate crops close to the soil, such as rice, and thence be transmitted to humans (Jensen et al., 2003). Although cooking rice above 55 °C can kill *B. cereus* cells, spores can survive cooking and then germinate and grow into bacteria when growth conditions are suitable. *B. cereus* were reported to be capable of forming biofilms (Alonso & Kabuki, 2019). The cooking temperature has main effect on B. cereus control (Albaridi & Yehia, 2021). Leaving cooked rice at room temperature, or storing it at an inappropriate temperature (4-55 °C) may allow bacteria to become active and multiply. Indeed, *B. cereus* spores grow faster in rice than in other food such as pasta and beans (Juneja et al., 2018). Thus samples of cooked rice have often been reported to be positive for *Bacillus cereus* (Forero et al., 2018). Temperatures between 25 and 35 °C are best for *B. cereus* growth (Ehling-Schulz et al., 2019), while the optimal temperature for the growth of spores in boiled rice has been shown to be between 30 and 37 °C (Gilbert et al., 1974) meaning that cooked rice left at room temperature in hotter climates presents a serious risk of food poisoning as *B. cereus* bacteria germinate and multiply. Although keeping rice cool can reduce bacterial growth, it needs to be reheated up to 60 °C before consumption to reduce the risk of *B. cereus* contamination (Guérin et al., 2017). The decimal reduction time, or the D-value (D95), for *B. cereus* at 95 °C ranged from 1.5 to 36.2 min in distilled water. These variations have been observed between different strains (Lake et al., 2004). In the study of Azanza & Centeno (2004) on a Philippine strain 1061 spores of B. cereus, it was found that the decimal reduction time D100 is 5 min. The literature states that the initial maximum concentration of spores in rice is 105/g. Therefore, the total heat inactivation time is determined as 25 min at 100 °C. When heated at this temperature and for this length of time, the rice will be overcooked. This is why the spores, which are present in uncooked rice and survive the usual heat treatment, can,

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under some conditions, survive in cooked rice and germinate into vegetative forms and then subsequently multiply and cause the food poisoning.

More than 2000 cases of food poisoning that have caused vomiting and/or diarrhea were reported in Saudi Arabia in 2018 (Saudi Arabia, 2018). Some outbreaks of food poisoning have been linked to eating rice cooked at home, in restaurants, hospital kitchens, camps, etc. In addition, *B cereus* is considered one of the major health risks that causes food poisoning every year during Hajj season (Memish et al., 2014). Some notable food poisoning incidents associated with rice in Saudi Arabia include an outbreak that happened at a wedding ceremony (Aljoudi et al., 2010), and one involving a number of soldiers who developed gastroenteritis and diarrhea after a meal of rice in the hot season (Al-Joudi, 2007). Furthermore Al-Abri et al. (2011) reported a case when more than 50 patients in a hospital had symptoms of poisoning approximately 14 hours after eating a rice meal contaminated with *B. cereus* for lunch. Such outbreaks can occur due to inadequate preparation and improper storage of rice (Memish et al., 2014). This is a particular risk after gatherings and parties since, often, large amounts of rice are left over and these may be stored at room temperature for a long time before refrigerating or transferring to charities. Such bad storage conditions invite *B. cereus* growth, and reheating cooked rice might not eliminate the bacteria if the rice is sufficiently contaminated before or after cooking and cooling (Kramer & Gilbert, 1989).

Given the concerns outlined above, this research investigates the possibility of food poisoning occurring due to the presence and growth of *B. cereus* and its spores in rice.

2 Materials and methods

2.1 Rice sample collection

This study was conducted to check the prevalence of *Bacillus cereus* bacteria and its spores in cooked and uncooked rice samples. A total of forty rice samples were purchased from local supermarkets in 2020 (all imported, and among the brand most often purchased by customers around Saudi Arabia according to the supermarket marketing divisions).

2.2 Enumeration of Bacillus cereus

The samples were examined under sterile conditions for the presence of *B. cereus*. Dry uncooked and cooked rice samples were tested. For the cooked samples, the rice was cooked at medium heat for 30 min then stored at room temperature for twelve hours to let bacteria multiply and spores grow. Using the colony count technique, duplicates of each (10 g) sample were added to 90 mL of sterile pure water in sterile filter bags then homogenized. *B. cereus* was detected by plating dilutions on petri dishes with selective media, mannitol egg yolk polymyxin (MEYP) agar, as described in International Organization for Standardization (2004). The biochemical tests were used to confirm that the isolates are *B. cereus* bacteria.

2.3 Determination of the D- and Z-values of B. cereus *ATCC 14579 spores in boiled rice*

The D-value was calculated from the slope of the regression line obtained with the values of the heat survival curves. The survival curves were obtained by plotting the log10 numbers of the surviving spores against the heating time (min) at each temperature, modified method of Stumbo (1973). The experiments were carried out in 50 × 15 mm screw-capped test tubes containing 10 mL of boiled rice. Each test tube contained 5 mL of the *B. cereus* spore suspension and was inoculated to obtain 108 spores/ml. The inoculated were heated in a thermostatically controlled water bath (Memmert GmbH+, West Germany). Thermal resistance was determined at temperatures of 80, 85, 90, 95, and 100 °C at different interval times of 0, 1, 2, 3, and 4 min. Replicate test tubes were removed immediately and immersed in an ice water bath. After cooling, 1, 0.1, and 0.01 mL of the appropriate dilutions were placed into petri dishes and mixed with a poured nutrient agar medium. The plates were incubated at 37 °C for 24 h and the number of survivors was determined by counting the CFUs/ml. D-value curves were constructed by plotting the logarithm number of the surviving cells against the heating time. The D-values of the B. *cereus* spores in boiled rice broth were based on the time required for the survivor curves to reach a reduction of 1 logarithmic cycle (Jay, 1992; Mossel et al., 1995). This D-value is numerically equal to the time required to achieve 90% destruction of the treated bacterial population.

The Z-value was determined by plotting the log D-value means against the corresponding temperature. Thus, thermal resistance curves were constructed by plotting the logarithm of the D-values against the exposure temperature. The Z-value is the increase in temperature required for a curve reduction of 1 logarithmic cycle (Stumbo, 1973; Frazier & Westhoff, 1988). It is numerically equal to the temperature increase required to decrease the D-values by 90%.

3 Results and discussion

B. cereus was observed in 50% of the raw rice samples, as shown in Table 1. This result is in accordance with that of Yu et al. who found that 50% of rice/noodle samples were *B. cereus* positive (Yu et al., 2020). Sidiqui et al. (2019) found that 25% of rice

Table 1. *Bacillus cereus* count in uncooked and cooked rice samples.

Sample	Number of samples	Not detected	<100 cfu/g	< 10^3 cfu/g	< 10^4 cfu/g*	>10^4cfu/g*
Row	40	20 (50%)	19 (47.5%)	1(2.5%)	0 (0%)	0 (0%)
Cooked	40	40 (100%)	0 (0%)	0(0%)	0 (0%)	0 (0%)
Cooked and stored for 12 hours	40	38 (95%)	0 (0%)	0(0%)	2(5%)	0 (0%)

*GSO limits.

samples were *B. cereus* positive, while Forero et al. found that 9% of the rice samples that they tested were positive. Immediately after cooking no growth of *B. cereus* was observed due to the effect of heat. After the cooked rice had been allowed to cool it was stored at room temperature for twelve hours. The samples were then reanalyzed, with two showing *B. cereus* growth of 4×10^3 and 6×10^3 Cfu/g.

The allowed limits for *B. cereus* count for starch-rich foods vary. In Europe up to 10^5 Cfu/g is allowed, but according to the Gulf Cooperation Council (GCC) standardization organization (GSO), the limit in the GCC is 10^4 Cfu/g (Gulf Cooperation Council, 2015). Although 10^5 Cfu/g is the lowest concentration found to cause *B. cereus* poisoning in adults, (10^3 Cfu/g) have been found to cause diarrheal illness in infants and the elderly. None of the positive samples in this study exceeded the GSO limits, similar to Sidiqui's results, where most of the count in positive samples was below 10^5 cfu/g (Sidiqui et al., 2019) .

The D- of *B. cereus* ATCC 14579 spores in heated rice are shown in Figure 1. The D-values at 80, 85, 90, 95, and 100 °C were 2.7, 1.55, 1.35, 1.2, and 0.6 min, respectively. Figure 2 shows the Z-value for *B. cereus* ATCC 14579 at 9 °C.

The results of this study are in agreement with those of Sarrías et al. (2002), who calculated the D95-values in raw rice to range from 0.7 to 5.2 min. In another comparative study carried out by Parry & Gilbert (1980), it was reported that the D95-values range from 2.5 to 36.2 min for strains isolated from emetic foodborne illness outbreaks (Parry & Gilbert, 1980), while Johnson et al. (1982) published D95-values from 1.2 to 20.2 min for spores of *B. cereus* strains found from diarrheal, emetic, and a toxigenic origins . Moreover, Sarrías et al. (2002) determined the D-values at 100 °C, which were shown to range from 0.45 to 0.99 min. These D100-values are in agreement with those obtained by Mazas et al. (1995) for the *B. cereus* strains ATCC 4342 and ATCC 7004; the strain ATCC 9818 was more heat resistant (D100 > 44 min).

For Z-values result, Our result in agreement with the value obtained by Sarrías et al. (2002), who determined the Z-values for strains of *B. cereus* tested in their study, which were shown to range from 7.42 to 8.20 °C, with an average of 7.7 °C . The Z-values obtained by Mazas et al. (1995) for the three strains studied were very similar, ranging from 7.24 to 8.27 °C, with an average of 7.64 °C. Meanwhile, in another study, Gilbert et al. (1974) found that the Z-values ranged from 6.7 to 8.3 °C, and Johnson et al. (1982) reported Z-values of 6.8-13.9 °C.

Since room temperature can reach 37 °C in hot countries, conditions are often suitable for bacterial spores to regrow (Gilbert et al., 1974). In addition, *B. cereus* spores are heat resistant, an ability that becomes higher when fat is added to rice (Gilbert et al., 1974). Both these features mean that cooked rice left over from parties etc. is at very high risk of *B. cereus* growth increasing to dangerous levels, especially given that low temperature in reheating lower than 80 °C will not kill bacteria in contaminated rice. Accordingly, food quality control laboratories in Saudi Arabia should institute regular *B. cereus* checks. In addition, Saudi Arabia should initiate public health education

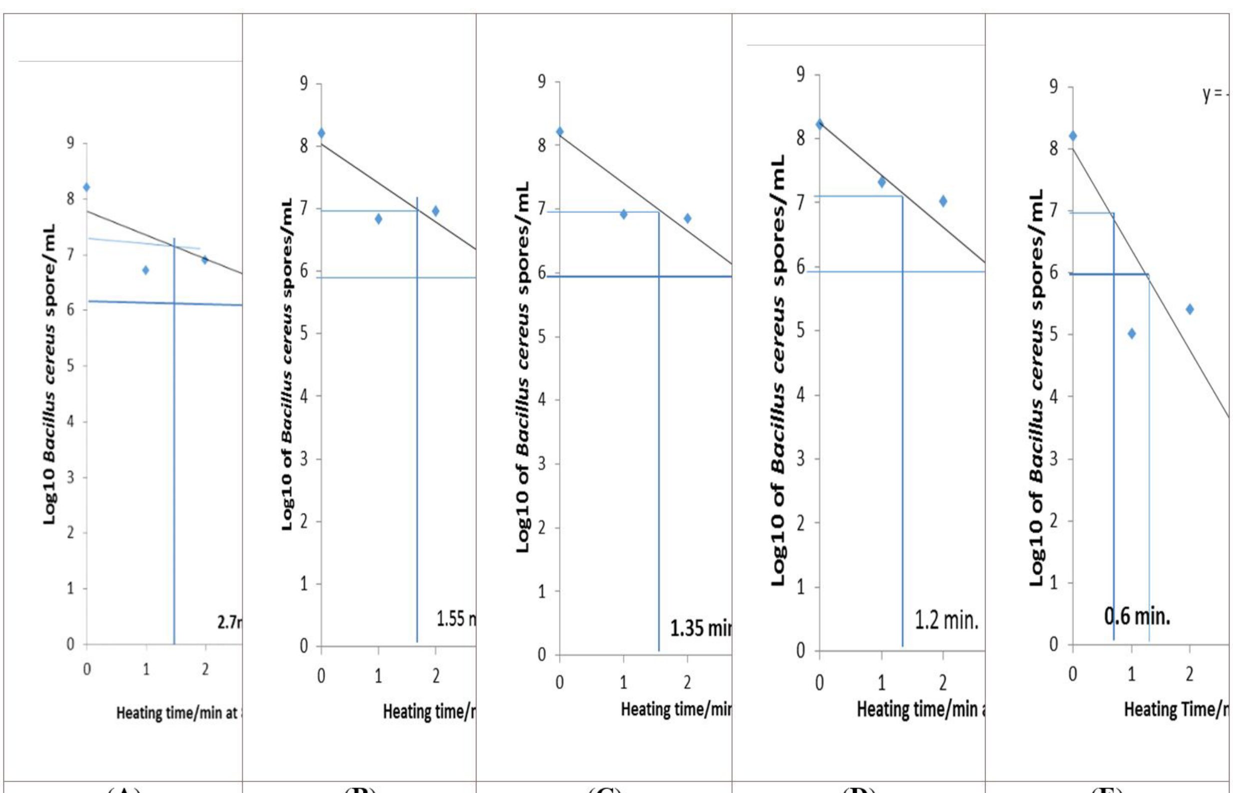

Figure 1. (A-E) *D*-values of *B. cereus* ATCC 17549 spores in heated rice at 80, 85, 90, 95, and 100 °C.

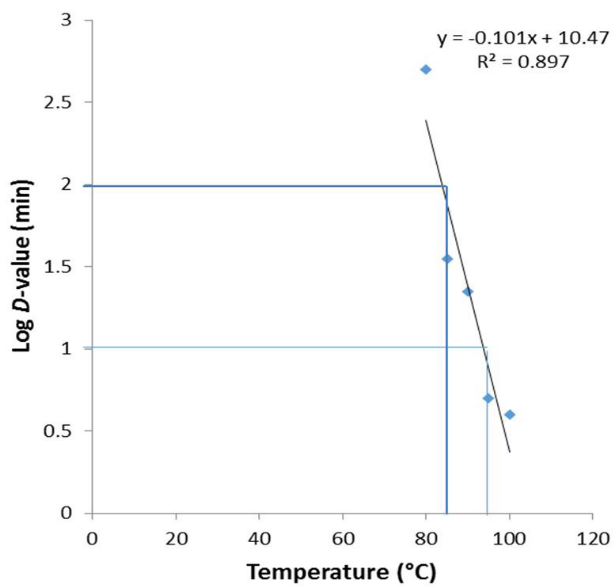

Figure 2. *Z*-value of *B. cereus* ATCC 14579.

programmes to enhance knowledge of food safety and storage routines in respect to rice, especially leftover rice, so as to reduce the risk of foodborne disease. In particular, education should focus on discouraging the culture of preparing excess amounts of rice such that the quantity of leftovers cannot be stored and refrigerated safely.

4 Conclusion

Daily consumption of large amounts of rice in Saudi Arabia encourages leftovers and poor storage routines, increasing the risk of illness from *B. cereus* poisoning. Exposing cooked rice to a wide range of temperature during storage might support the multiplication of bacteria in contaminated food. As rice is a major host for *B. cereus*, food quality control should include routine checks for *B. cereus* in both cooked and uncooked rice.

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